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Photoacoustic imaging of the near-infrared fluorescent protein iRFP *in vivo*

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Abstract:

Genetically encoded probes powerfully and non-invasively target specific tissues, cells, and subcellular locations. iRFP, a novel near-infrared fluorescent protein with low quantum yield whose absorption and fluorescence maxima are located at wavelengths longer than the Q-band of hemoglobin absorption, is ideal for PAT. Here, we report on an *in vitro* comparison of iRFP with other far-red fluorescent proteins, and its use in imaging a mouse tumor xenograft model. In an *in vivo* experiment, we stably transfected iRFP into MTLn3 adenocarcinoma cells and injected them into the mammary fat pad of female SCID/NCr mice, then imaged the resulting tumors two and three weeks post injection. The contrast increase from the protein expression was high enough to clearly separate the tumor region from the rest of the animal.

Keywords: iRFP, fluorescent proteins, photoacoustic tomography, genetically encoded probe, deep imaging

Introduction:

Genetically encoded fluorescent proteins (FPs) can provide endogenous contrast for various imaging applications since they can be produced by living cells and tissues. The application of conventional FPs for PA imaging of mammals has been a problem due to high hemoglobin absorption below 650 nm[1]. iRFP has a very high extinction coefficient of 105,000 $M^{-1}cm^{-1}$, a low fluorescence quantum yield of 6%, and its absorption spectra lies in the near-infrared window, all beneficial properties for PA imaging[2]. iRFP, being a fluorescent protein, also provides additional options for signal analysis and quantification through fluorescence.

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Results:

We wanted to compare the relative signal of each of the currently available far-red FPs to that of iRFP. We can see in figure 1 that blood has higher contrast at shorter wavelengths, however as the wavelength increases, due to its high extinction coefficient and favorable spectra, iRFP has the highest signal ratio to blood of the tested FPs. In these images, negative values represent stronger signal.

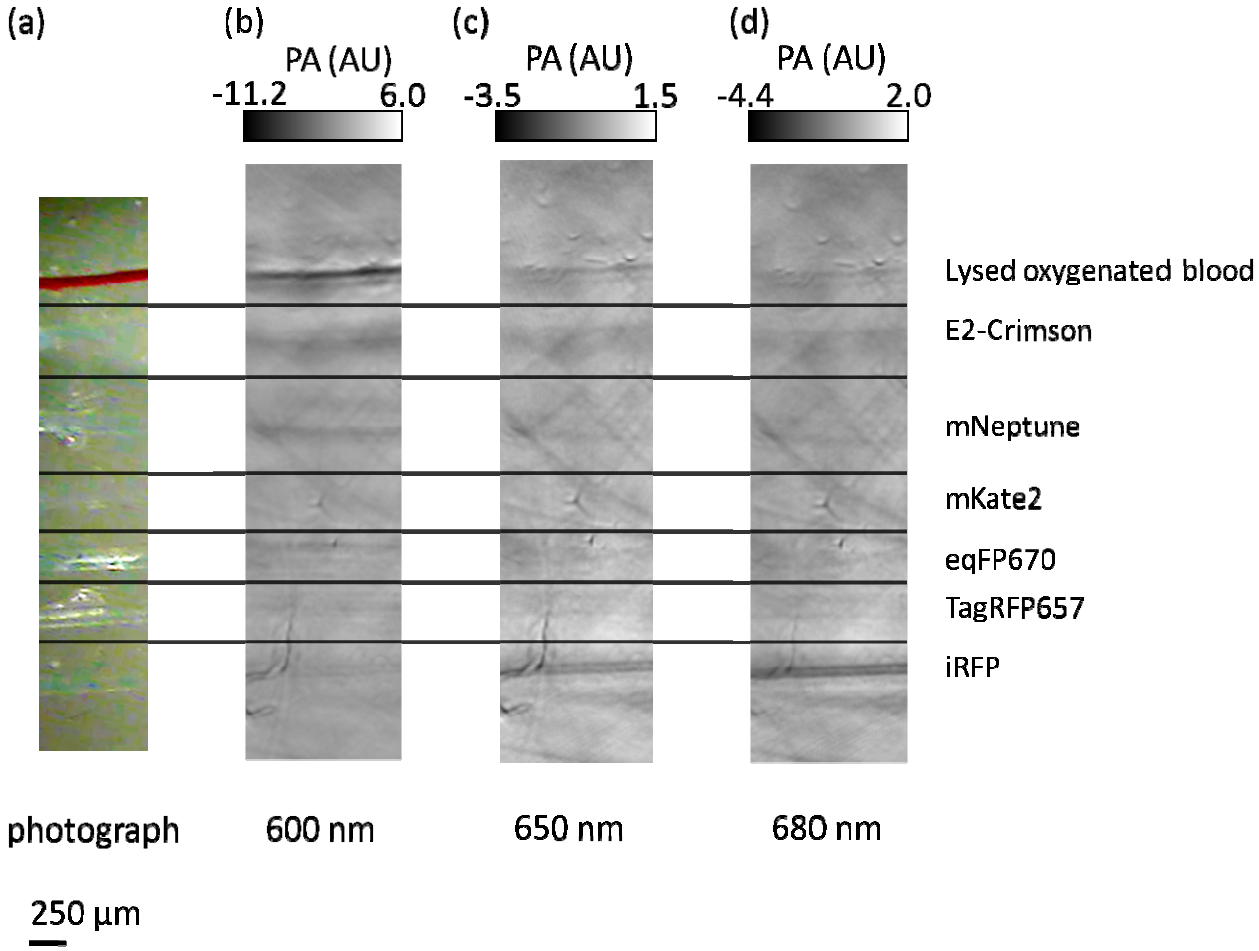

Figure 1: (a) Photograph of Silastic tubing samples. PA images of purified iRFP and far red GFP-like proteins along with blood for comparison at 600 nm (b), 650 nm (c), and 680 nm (d).

We also performed an *in vivo* experiment where MTLn3 adenocarcinoma cells were stably transfected to express the iRFP protein under a constituently on cmv promoter. About one million cells were then injected in a SCID/NCr female mouse mammary fat pad. The resulting tumors were then imaged two weeks after the injection using a photoacoustic computed tomography (PACT) system consisting of a 512 element full ring transducer as shown in figure 2.

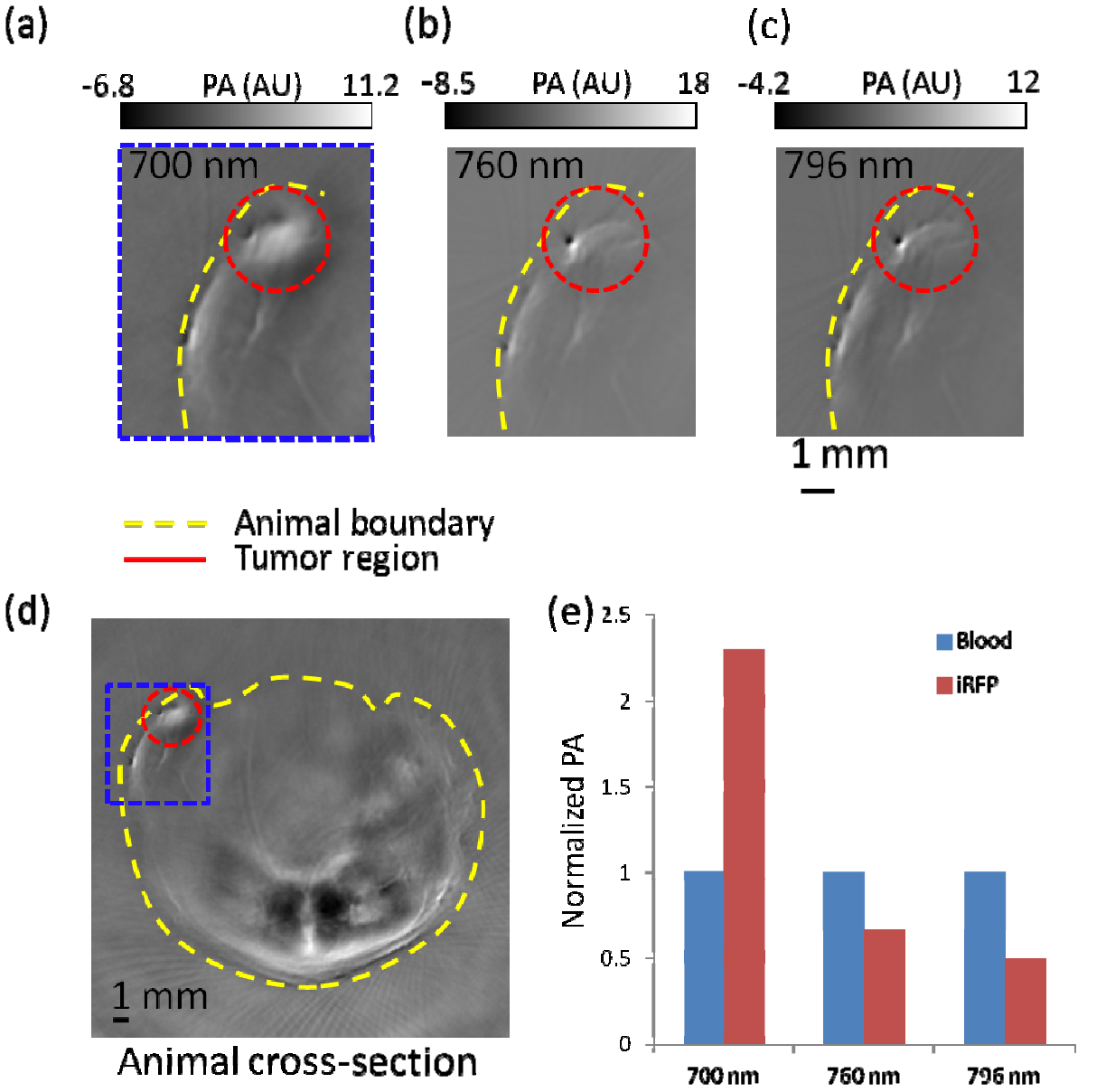

Figure 2: (a)-(c) PACT mouse cross-section images taken using the indicated wavelengths. (d) Animal cross-section area shown for reference of tumor position. The yellow dashed line indicates the mouse border, blue dashed box represents the zoomed regions above, and red dashed circle represents the tumor region. (e) Relative concentration recovered from tumor region normalized by blood signal.

A further advantage of using fluorescence based contrast agents for photoacoustic imaging is the ability to confirm protein expression and spatial distribution using conventional fluorescence imaging. In this case we show a planar white light image with corresponding fluorescence image in figure 3.

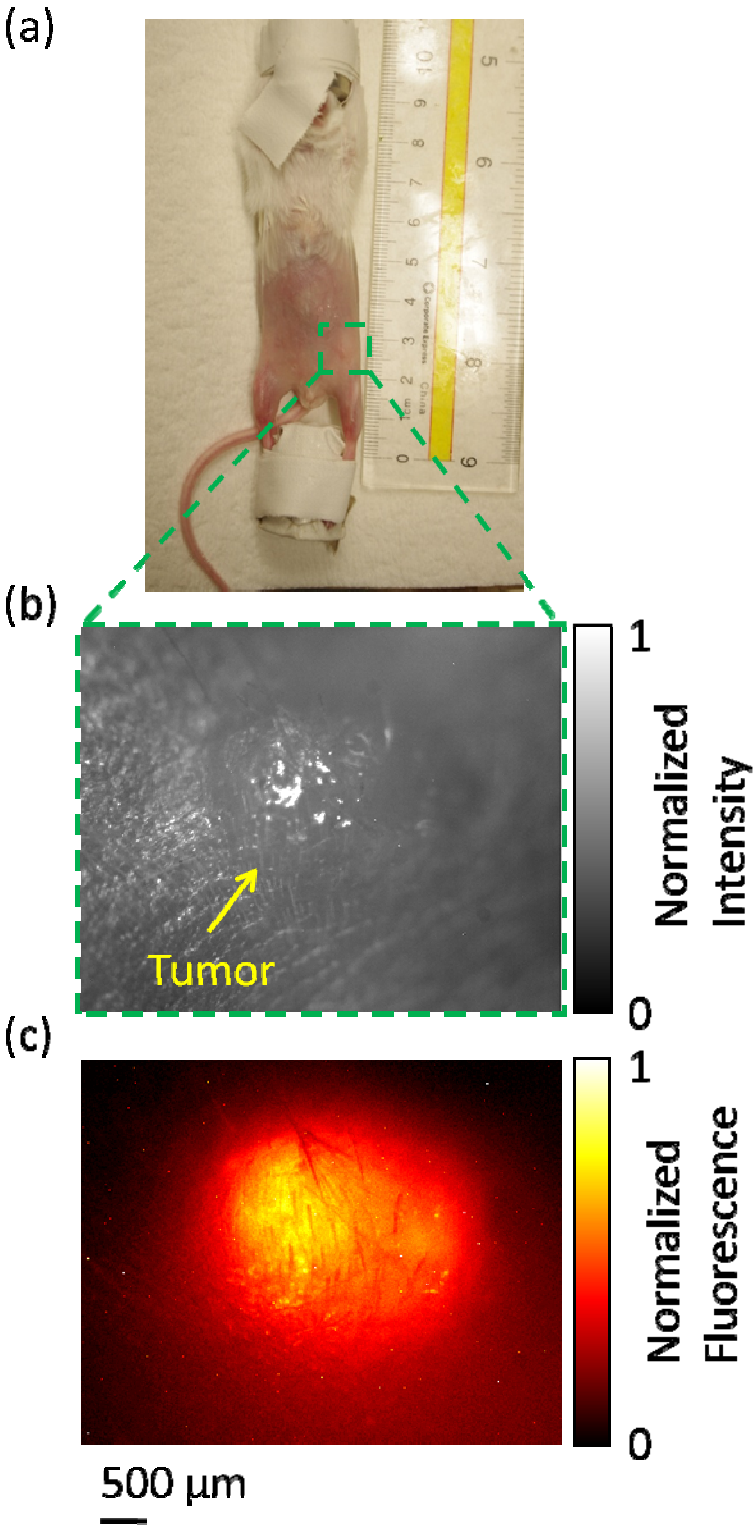

Figure 3: Fluorescence confirmation. (a) White light photograph showing mouse with green box showing tumor region. (b) White light image showing tumor. (c) Normalized planar fluorescence of tumor area.

Until recently, excitation of FPs was limited to the blue, green, and red spectral regions where hemoglobin absorption is high. Because of this, their use for whole-body imaging has been challenging. By combining an iRFP probe with the deep imaging capabilities of PACT this study demonstrates the capability of deep tissue imaging of fluorescent proteins.

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